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Screening *Solanum* germplasm (Tomato and Brinjal) and Identify Rootstocks for Resistance to Bacterial Wilt

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ABSTRACT: *Ralstonia solanacearum* is a soil-borne bacterial pathogen and a major limiting factor in the production of many crop plants around the world. The present study was conducted in *Rabi*, 2021 at the Postgraduate Research Block, Department of Vegetable Science, College of Horticulture, Sri Konda Laxman Telangana State Horticultural University, Rajendranagar, Hyderabad. The current study examined 25 genotypes for Bacterial wilt resistance at the morphological level. Twenty-five genotypes of tomato and brinjal including susceptible checks (Arka Vikas and Pusa ruby) were screened under artificial inoculation conditions in poly bags. Out of 25 genotypes, three were found highly resistant (HR) *viz.,* Arka Keshav, Surya, *Solanum torvum*, one genotype *i.e.* LA -0490 is resistant (R), two genotypes *i.e.* EC-631379, LA-1589 were recorded moderately resistant (MR),one genotype *i.e.* EC-620509 is moderately susceptible (MS), fourteen genotypes *viz.,* EC-620428, EC-620378, EC-620389, EC-620394, EC-631369, EC-620503, AVTO-9803, EC-620452, Money maker, Marutham, Pusarohini, Pusasheethal, PKM-1 and Arka Vikas were susceptible (S) and four genotypes *i.e.* EC-615055, EC-620422, EC-620441 and Pusa ruby were recorded highly susceptible (HS) to bacterial wilt. Genotypes exhibiting Bacterial wilt resistance will be grafted on to commercial variety of tomato and evaluated under open field conditions for yield, quality parameters and disease resistance under Telangana conditions.

Keywords: Tomato, Brinjal, Bacterial wilt, Genotypes.

INTRODUCTION

In India, tomato and brinjal cultivation is severely affected by the bacterial wilt caused by *Ralstonia solanacearum* (Smith) (Yabuuchi *et al*., 1992). It is reported to be among the top five diseases (Elhinstone, 2005) and is a major yield constraint of brinjal in coastal region of India (Ramesh *et al.*, 2016). The disease is soil-borne and the pathogen invades the host through wounds in roots or underground parts of the plant. The lower leaves of the plants droop and show partial wilting. The plants suddenly collapse and die in a day or two. The death of the plants is seldom accompanied by chlorosis of the leaves. The management of this pathogen is difficult due to the presence of diverse *R. solanacearum* strains and the ability of the pathogen to survive longer even in adverse soil conditions. Different management strategies *viz.,* resistant varieties (Dalal *et al*., 1999), soil amendments (Islam and Toyota 2004), soil solarization (Kumar and Sood 2001), use of bio-fumigants (Pradhanang *et al*., 2003), transgenic resistant plant (Jia *et al*., 1999), plant growth promoting rhizobacteria (Guo *et al*., 2001; Singh *et al*., 2012), use of systemic acquired resistance (SAR) inducers (Anith *et al.*, 2004), biological control (Ramesh and Phadke 2012; Achari and Ramesh 2014), had been developed with limited success in the bacterial wilt management.

MATERIALS AND METHODS

The experimental material consisted of 15 germplasm lines, 9 released varieties and 1 wild species (Table 1)

obtained from NBPGR, Regional Station, Hyderabad; IARI, New Delhi; IIHR, Bengaluru; TNAU, Periyakulam; UC DAVIS, California, USA; TNAU, Coimbatore; KAU, Thrissur and COE, Jeedimetla. All cultivars were systematically evaluated during the research period. The experiment was presented using 25 tomato and brinjal genotypes in a randomized block design (RBD) with three replicates during Rabi, 2021 at PG Student Research Block, College of Horticulture, Rajendranagar, Hyderabad. Tomato and Brinjal seeds were sown in portrays filled with coco-peat and watered regularly. 21 days old healthy seedlings were used for inoculation. Before inoculation, the plants were starved for 24 hours by avoiding watering. *Ralstonia solanacearum* infected plants were collected from Noble seeds private limited, Bengaluru. The presence of pathogen was tested by placing longitudinal sections containing vascular tissue from diseased plants in a test tube with distilled water. The infested tissue showed fine streaks of milky ooze and composed of masses of bacteria which come out from the margin of the cut portion within a few minutes. This distinguishes *Ralstonia* wilt from other wilts like fusarium wilt and physiological wilt. The ooze-out was plated on Triphenyl Tetrazolium Chloride (TTC) media. Bacterial inoculum was prepared from the virulent (pink-colour at centre, irregular shaped and mucoid) colonies, grown in Triphenyl Tetrazolium Chloride (TTC) media. The composition of Triphenyl Tetrazolium Chloride (TTC) media is shown in (Table 2) as follows:

It is recommended that the inoculation test be performed as soon as possible after the medium has been prepared. The solution is autoclaved for at least 15 minutes at 121°C. Then, cool it to 50-60°C and add 5ml of 1% TTC solution (50 mg/5 ml) sterilised with micro-litre (0.2 m) filter. Extra TTC solution can be kept in the refrigerator at 4 °C for up to a year. When the media is ready, culture the inoculum in the plate by dipping the tip of the spreader into the inoculum and streaking it on the plate for 48 hours. Pinkish red centred colonies with rough edges and cream colour on the edge were observed on TTC medium, which is typical of *R. solanacearum.* Colonies with smooth edges and no cream colour on the edge are non-virulent and are not good for inoculation. For the pathogenicity test single colony of RS showing virulent, fluidal, irregular and creamy white colour was selected for each group of isolates and multiplied in a NA medium. Add freshly grown *R. solanacearum* colonies to 50 ml NA in a sterile vial with a sterile loop and allow to grow in a shaking incubator maintained at 28 °C and 150 rpm for 24 h. Before inoculation, the roots were slightly severed by inserting a sharp knife 1.0 cm away from the stem. Root severing was done to ensure bacterial penetration through roots.

Procedure for inoculation: (Patrick juma, 2018). Uproot the seedlings when they have 2-3 true (main) leaves after 3-4 weeks and lightly wash the roots with tap water in a batch of about 20 seedlings. Place the seedlings in a beaker or cup. Pour 100ml of prepared bacterial wilt solution with a specific concentration (4×10^8cfu/ml inoculum) into the cup (beaker). Dip the tomato and brinjal seedlings roots in the bacteria suspension to inoculate them. The inoculated seedlings are transplanted into pots with soil that is not contaminated (infected) with disease-causing microorganisms. The inoculated seedlings are then grown and the degree of wilting of each seedling is observed. The bacterial wilt symptoms should be observed in a period of 2-4 weeks depending on the cultivar and weather factors particularly atmospheric temperature.

Disease scoring. The number of wilted plants in each accession recorded and classified into five different groups according to Winstead and Kelman (1952).

Disease reaction	Percentage of wilt incidence
Highly Resistant (HR)	Plants do not show any wilt symptom
Resistant (R)	1-20 % wilted plants
Moderately Resistant (MR)	21-40 % wilted plants
Moderately Susceptible (MS)	41- 60 % wilted plants
Susceptible (S)	61-80 % wilted plants
Highly Susceptible (HS)	More than 80 % wilted plants

Per cent disease incidence. The Per cent disease incidence was calculated by using following formula.

Per cent Disease Incidence (PDI) %

$$= \frac{\text{Number of infected plants}}{\text{Total number of plants observed}} \times 100$$

RESULT AND DISCUSSION

The percent disease incidence in genotypes was ranged from 0% to 93.33%. Arka Keshav, Surya, *Solanum torvum* showed high resistance against *Ralstonia solanacearum* and exhibited 0.00% disease incidence respectively and categorized under the reaction group highly resistant (HR).The per cent disease incidence reported in the genotype LA -0490 is 13.33% and categorized under the reaction group resistant (R) and the genotypes EC-631379 and LA-1589 recorded 33.33% incidence respectively and categorized under the reaction group moderately resistant (MR), EC-620509 showed 46.66% disease incidence and categorized under the reaction group moderately susceptible (MS). Genotypes *viz.,* EC-620428, EC-620378, EC-631369, AVTO-9803, EC-620452, Money maker, Marutham, Pusasheethal, PKM-1 recorded 66.66% disease incidence respectively and EC-620389, EC-620394, EC-620503, Pusarohini, and Arka Vikas recorded 73.33% incidence and categorized under the reaction group susceptible (S). Genotypes EC-620422, EC-620441, Pusa ruby showed 93.33% incidence and EC-615055 showed 86.66% incidence respectively and categorized under the reaction group Highly susceptible (HS) (Table 3).

Present findings were corroborated with the findings of Mochizuki and Yamakawa (1979) observed that *Solanum torvum* showed potential root stock because of high resistance to bacterial wilt and it help to increase good fruit yield of the scion. Shetty and Reddy (1985); Ali *et al.* (1990) recorded that *S. sisymbrifolium* and *S. torvum* are the effective rootstock to control bacterial wilt. Ali, (1993); Rahman *et al.* (2002) reported *Solanum torvum* as resistant rootstock against bacterial wilt. Chaudhary and Sharma (2000) observed that the genotypes Arka Keshav, Arka Neelkanth, Arka Nidhi and SM 6-6 were observed to be resistant to bacterial wilt.As noted by Singh and Malhotra (2010) Arka Keshav and Arka Anand (F1 hybrid) are commercially available resistant varieties against *Ralstonia solanacearum.* Superiority of *Solanum torvum* rootstock against bacterial wilt infestation caused by *Ralstonia solanacearum* may be observed inline of the work concluded by Aribaud *et al.* (2014) who observed, an increased cell wall mono amine oxidase activity in *Solanum torvum* after *Ralstonia solanacearum* inoculation.

Kumar *et al.,* (2014) revealed that among the accessions of brinjal evaluated Arka Nidhi was found most resistant. Sahoo (2015) screened five different brinjal germplasms against bacterial wilt disease, out of which Utkal Tarini, Utkal Madhuri, Utkal Jyoti, Utkal Anushree showed high resistance. Santhosha *et al.* (2015) screened 40 brinjal genotypes for bacterial wilt resistance. Among them, Arka Nidhi, was found to be resistant to bacterial wilt whereas, Pusa Hybrid-6, Arka Shirish, R-2585 and R-2583 were found to be highly susceptible to bacterial wilt. Biswas and Ghosh (2018) observed minimum disease incidence (7.86 %) in Surya variety and categorized under resistant group. Bhanwar *et al.* (2019) investigated a few brinjal cultivars and hybrids grown in artificially inoculated soil in pot culture. Among them, Pusa Kranti was found to be moderately resistant. Similar results were noticed by Reshmi upreti and Pious Thomas (2015); Manoj *et al.* (2019). Bhattacharjee *et al.* (2022) screened twenty-five brinjal genotypes for bacterial wilt resistance under artificial and field conditions. Among them, 'Utkal Anusree' found to be resistant to bacterial wilt.

Table 1: List of genotypes used for disease screening along with their sources.

Sr. No.	Name of the Variety/Accession	Source
1.	EC-620509	NBPGR, Hyderabad
2.	EC-615055	NBPGR, Hyderabad
3.	EC-620428	NBPGR, Hyderabad
4.	EC-620378	NBPGR, Hyderabad
5.	EC-620389	NBPGR, Hyderabad
6.	EC-620394	NBPGR, Hyderabad
7.	EC-620422	NBPGR, Hyderabad
8.	EC-631369	NBPGR, Hyderabad
9.	EC-620503	NBPGR, Hyderabad
10.	AVTO-9803	NBPGR, Hyderabad
11.	EC-631379	NBPGR, Hyderabad
12.	EC-620441	NBPGR, Hyderabad
13.	EC-620452	NBPGR, Hyderabad
14.	LA-1589	UC, DAVIS, California, USA
15.	LA -0490	UC, DAVIS, California, USA
16.	Money maker	UC, DAVIS, California, USA
17.	Marutham	TNAU, Coimbatore
18.	Pusarohini	IARI, New Delhi
19.	Pusasheethal	IARI, New Delhi
20.	Pusa ruby	IARI, New Delhi
21.	PKM-1	TNAU, Periyakulam,
22.	Arka Vikas	IIHR, Bengaluru
23.	Arka Keshav	IIHR, Bengaluru
24.	Surya	KAU, Thrissur
25.	*Solanum torvum*	COE, Jeedimetla

EC: Exotic collection

Table 2: Composition of Triphenyl Tetrazolium Chloride media (for 1 litre) at pH: 7.

Sr. No.	Composition	Quantity
1.	Peptone	10g
2.	Agar	18g
3.	Casein	1g
4.	Glucose	5g
5.	Distilled water	1lit
6.	2,3,5-Triphenyl tetrazolium chloride	50mg

Table 3: Percent disease incidence and disease reaction of bacterial wilt in 25 genotypes.

Sr. No.	Name of the genotypes	Percent disease incidence (%)	Disease reaction
1.	EC-620509	46.66	MS
2.	EC-615055	86.66	HS
3.	EC-620428	66.66	S
4.	EC-620378	66.66	S
5.	EC-620389	73.33	S
6.	EC-620394	73.33	S
7.	EC-620422	93.33	HS
8.	EC-631369	66.66	S
9.	EC-620503	73.33	S
10.	AVTO-9803	66.66	S
11.	EC-631379	33.33	MR
12.	EC-620441	93.33	HS
13.	EC-620452	66.66	S
14.	LA-1589	33.33	MR
15.	LA -0490	13.33	R
16.	Money maker	66.66	S
17.	Marutham	66.66	S
18.	Pusarohini	73.33	S
19.	Pusasheethal	66.66	S
20.	Pusa ruby	93.33	HS
21.	PKM-1	66.66	S
22.	Arka Vikas	73.33	S
23.	Arka Keshav	0.00	HR
24.	Surya	0.00	HR
25.	*Solanum torvum*	0.00	HR
	S.Em±	**5.75**	
	SD	**28.79**	

CONCLUSIONS

Results from bacterial wilt screening revealed that Arka Keshav, Surya, *Solanum torvum* showed high resistance (HR), LA -0490 is resistant (R) and two genotypes *i.e*. EC-631379, LA-1589 were recorded moderately resistant (MR) against *Ralstonia solanacearum.*

FUTURE SCOPE

Bacterial wilt is a devastating disease of tomato and brinjal that causes high yield losses in fields and commercial greenhouses, implying the need for disease resistance development. The identified genotypes can be tested further and used in Marker Assisted Selection or gene pyramiding programmes to develop disease resistant commercial cultivars.

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Conflicts of Interest. None.

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